# Lipoxygenase Inhibitory Tetraketones: Potential Remedial Source for Inflammation and Asthma

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## *ABSTRACT*

***Objective:*** *A series of tetraketones has been synthesized by way of a one pot synthesis and screened for inhibitory activity against the enzyme lipoxygenase.*

***Method:*** *An efficient and high yielding one pot synthesis to tetraketones* ***[2–22]*** *has been developed by way of tetraethyl ammonium bromide ($Et_4N^+Br^-$) mediated condensation of cyclohexane-1, 3-dione* ***[1]*** *with a variety of aldehydes. Lipoxygenase enzyme solution was prepared so that enzyme concentration in reaction mixture was adjusted to give rates of 0.05 absorbance/minute. The test compounds were prepared in methanol of concentrations 50, 25, 12.5, 6.25 and 3.125 µM. The reaction mixture contained 160 µL (100 mM) sodium phosphate buffer (pH 8.0), 10µL of test-compound solution and 20µL of lipoxygenase solution. The contents were mixed and incubated for 10 minutes at 25°C. The reaction was then initiated by the addition of 10µL substrate solution (linoleic acid, 0.5 mM, 0.12% w/v tween 20 in the ratio of 1:2), with the formation of (9Z,11E)-(13S)-13-hydroperoxyoctadeca-9,11-dienoate, the change of absorbance at 234 nm was followed for 6 minutes.*

*The concentrations of the test compounds that inhibited the lipoxygenase activity by 50% ($IC_{50}$) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The $IC_{50}$ values were calculated by means of the EZ-Fit Enzyme-Kinetics Program (Perrella Scientific Inc., Amherst, USA).*

***Result:*** *The tetraketones* ***[2–22]*** *were synthesized in high yields (91–98%) using mild reaction conditions. Most of these compounds showed significant inhibitory activity against the enzyme lipoxygenase. It was found that the presence of substituents which increase delocalization of electrons enhances the inhibitory activity.*

***Conclusion:*** *It is concluded that the study is likely to lead to the discovery of therapeutically efficient agents against important disorders such as inflammation and asthma.*


# Las Tetracetonas Inhibidoras de la Lipoxigenasa: Fuente Potencial Remedial para la Inflamación y el Asma

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## *RESUMEN*

***Objetivo:*** *Una serie de tetracetonas han sido sintetizadas mediante síntesis de varios pasos en un solo reactor (one-pot), y examinadas en relación con su actividad inhibitoria frente a la enzima lipoxigenasa.*

***Método:*** *Una síntesis one-pot de un rendimiento alto y eficiente para la obtención de tetracetonas* ***(2-22)*** *ha sido desarrollada mediantebromuro amónico tetraetílico ($Et_4N^+Br^-$) – deciclohexano-1,3-diona, con una variedad de aldehídos. La solución de enzima lipoxigenasa fue preparada de modo que la concentración de la enzima en las mezcla de la reacción fue ajustada para que diera tasas de 0.05 absorbancia/minuto. Los compuestos de la prueba fueron preparados en metanol de concentraciones (50, 25, 12.5, 6.25 y 3.125 µM). La mezcla de reacción contenía 160 µL (100 mM) de un tampón (buffer) de fosfato de sodio (pH 8.0), 10µL de solución de compuesto de prueba, y 20µL de solución de*


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*lipoxigenasa. Los contenidos fueron mezclados e incubados por 10 minutos a 25°C. La reacción fue iniciada entonces por la adición de 10µL de solución substrato (ácido linoleico, 0.5 mM, 0.12% p/v tween 20 en proporción de 1:2), con la formación de (9Z, 11E)-(13S)-13-hidroperoxioctadeca-9,11-dienoato, el cambio de absorbancia a 234 nm fue seguido por 6 minutos. Las concentraciones de los compuestos de prueba que inhibían la actividad de la lipoxigenasa en un 50% ($IC_{50}$) fueron determinadas monitoreando el efecto del aumento de las concentraciones de estos compuestos en los ensayos sobre el grado de inhibición. Los valores $IC_{50}$ fueron calculados mediante el Programa Cinética de la Enzima EZ-Fit (Perrella Scientific Inc., Amherst, USA).*

***Resultados:*** *Las tetracetonas **(2-22)** se sintetizaron con elevados rendimientos (91–98%) usando condiciones de reacción leve. La mayoría de estos compuestos mostraron una actividad inhibitoria significativa frente a la enzima lipoxigenasa. Se halló que la presencia de sustituyentes que aumentan la deslocalización de los electrones contribuye a mejorar la actividad inhibitoria.*

***Conclusión:*** *Se concluye que es probable que el estudio conduzca al descubrimiento de agentes terapéuticamente eficientes frente a trastornos importantes tales como la inflamación y el asma.*

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## INTRODUCTION

Tetraketones are important compounds which are extensively used as precursors for the synthesis of acridinediones as laser dyes of various heterocyclic compounds (1). Previously, these target compounds have been prepared by several methods but most of these involve forcing reaction conditions, tedious reaction workups and involvement of heat to afford the products (2, 3). Herein, we report an efficient and high yielding one pot synthesis to these potential compounds involving tetraethyl ammonium bromide mediated condensation of cyclohexane 1, 3-dione **[1]** with a variety of aldehydes in water at room temperature. The resulting tetraketones **[2–22]** were obtained in a yield ranging from 91%–98%.

Lipoxygenases (LOX; EC 1.13.11.12) constitute a family of non-haeme iron containing dioxygenases that are widely distributed in animals and plants. In mammalian cells these are key enzymes in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acids (HETEs), leukotrienes, lipoxins and hepoxylines (4). It has been found that these lipoxygenase products play a role in a variety of disorders such as inflammation (5) and bronchial asthma (6). LOXs are therefore potential target for the rational drug design and discovery of mechanism-based inhibitors for the treatment of inflammation, bronchial asthma, cancer and autoimmune diseases. Most of the tetraketones obtained in the present study showed potential inhibitory activity against LOX.

## MATERIALS AND METHODS

### General procedure for the preparation of tetraketones, 2–22

To a mixture of cyclohexane-1,3-dione **[1]** (10 mmol), ammonium chloride (10 mmol), tetraethyl ammonium bromide (0.5 mmol) dissolved in water, aldehyde (5 mmol) was added drop-wise and the reaction mixture stirred at room temperature for 30 min and the reaction monitored with Thin Layer Chromatography (TLC). Cold water (15 mL) was then added to the reaction mixture and the resultant precipitates filtered and recrystallized from aqueous ethanol.

### *In Vitro* Lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel (7). Lipoxygenase enzyme solution was prepared so that enzyme concentration in the reaction mixture could be adjusted to give rates of 0.05 absorbance/minute. The test compounds were prepared in methanol of concentrations 50, 25, 12.5, 6.25 and 3.125 $\mu$M. The reaction mixture contained 160 $\mu$L (100 mM) sodium phosphate buffer (pH 8.0), 10$\mu$L of test-compound solution and 20$\mu$L of LOX solution. The contents were mixed and incubated for 10 minutes at 25°C. The reaction was then initiated by the addition of 10$\mu$L substrate solution (linoleic acid, 0.5 mM, 0.12% w/v tween 20 in the ratio of 1:2), with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9, 11-dienoate, the change of absorbance at 234 nm was followed for 6 minutes. The concentrations of the test compounds that inhibited the lipoxygenase activity by 50% ($IC_{50}$) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The $IC_{50}$ values were calculated by means of the EZ-Fit Enzyme-Kinetics Programme (Perrella Scientific Inc, Amherst, USA).

### Experimental

Melting points were determined using a Buchi 434 melting point apparatus. NMR spectroscopy was performed on a Bruker AMX NMR spectrometer. Infrared spectra (IR) were recorded on JASCO IR-A-302 spectrophotometer. EIMS were recorded on a FINNIGAN MAT-311A. Thin layer chromatography was performed on pre-coated TLC (Kieselgel 60, $F_{254}$, E. Merck, Germany). Chromatograms were visualized by iodine vapours, ultraviolet light (UV) at 254 and 365 nm, ceric sulphate solution followed by heating.

## RESULTS

### Phenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 2

White solid, m.p. 212-214 ºC; yield: 98%; TLC: Rf 0.58 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ 255 (log ε = 4.1) nm; IR (KBr): ν = 2950, 1662, 1510, 1343, 1452, 843 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): δ = 7.09–7.28 (m, 5H, Ar-*H*), 4.17 (m, 1H, H–7), 2.33-2.60 (m, 2H, H-2/H-2′), 2.09–2.30 (m, 8H, H–4/H–6/H–4′/H-6′), 1.97–2.01 (m, 4H, H-5/H–5′); EIMS: *m/z* 312 (25), 294 (6), 213 (20), 199 (70), 115 (50), 102 (80), 55 (100); $C_{19}H_{20}O_4$ (312.36): calcd. C 73.06, H 6.45; found: C 72.78, H 6.40%.

### 4-Methylphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 3

White solid, m.p. 192–194 ºC; Yield: 95%; TLC: $R_f$ 0.54 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 261 (log ε = 4.06) nm; IR (KBr): ν = 3110, 1615, 1608, 1513, 1475, 843 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.01 (d, *J* = 8.0 Hz, 2H, H–2″/H–6″), 6.92 (d, *J* = 8.0 Hz, 2H, H-3″/H–5″), 5.55–5.67 (m, 1H, H–7), 2.48–2.54 (m, 2H, H–2/H–2′), 2.02–2.11 (m, 8H, H–4/H–6/H–4′/H–6′), 2.26 (s, 3H, $CH_3$), 1.90–1.96 (m, 4H, H–5/H–5′); EIMS: *m/z* 326 (8), 296 (4), 242 (10), 213 (19), 199 (75), 115 (55), 84 (100), 55 (52); $C_{20}H_{22}O_4$ (326.38): calcd. C 73.60; H 6.79. found: C 73.12, H 6.67%.

### 2-Hydroxyphenyl -2,2′-methylenebis-(cyclohexane-1, 3-dione), 4

White solid, m.p. 230–232ºC; Yield: 94%; TLC: $R_f$ 0.48 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 288 (log ε = 4.0) nm; IR (KBr): ν = 3111, 1615, 1602, 1510, 1462, 1340, 844 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.15–6.87 (m, 5H, Ar-*H*), 5.15–5.28 (m, 1H, H–7), 2.44–2.55 (m, 2H, H–2/H–2′), 2.15–2.28 (m, 8H, H–4/H–6/H–4′/H–6′), 1.93–1.99 (m, 4H, H–5/H–5′); EIMS: *m/z* 328 (15), 310 (15), 254 (70), 253 (44), 199 (100), 171 (12), 170 (12), 115 (22); $C_{19}H_{20}O_5$ (328.35): calcd. C 69.50; H 6.14. found: C 69.42; H 6.04%.

### 3-Hydroxyphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 5

White solid, m.p. 160-162 ºC; Yield: 97%; TLC: $R_f$ 0.49 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 289 (log ε = 3.1) nm; IR (KBr): ν = 3159, 1612, 1605, 1515, 1450, 1340, 848 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.22 (t, *J* = 8.0 Hz, 1H, H–5″), 7.08 (d, *J* = 8.0 Hz, 1H, H–4″), 6.75 (dd, *J* = 8.0 Hz, *J* = 2.2 Hz, 1H, H–6″), 6.22 (d, *J* = 2.2 Hz, 1H, H–2″), 5.06–5.15 (m, 1H, H–7), 2.44–2.56 (m, 2H, H–2/H–2′), 2.14–2.25 (m, 8H, H–4/H–6/H–4′/H-6′), 1.91–1.97 (m, 4H, H–5/H–5′); EIMS: *m/z* 328 (5), 310 (17), 292 (20), 264 (10), 217 (70), 199 (20), 84 (36), 55 (100); $C_{19}H_{20}O_5$ (328.35): calcd. C 69.50; H 6.14. found: C 69.21; H 5.97%.

### 4-Hydroxyphenyl-2,2′-methylenebis-(cyclohexane-1, 3-dione), 6

White solid, m.p. 200-202 ºC; Yield: 98%; TLC: $R_f$ 0.51 (9:1 CH2Cl2/$CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 276 (log ε = 4.2) nm; IR (KBr): ν = 3112, 2945, 1645, 1505, 1456, 1333, 844; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.77 (d, *J* = 8.4 Hz, 2H, H–2/H–6), 7.55 (d, *J* = 8.4 Hz, 2H, H–3/H–5), 4.18 (m, 1H, H–7), 2.33–2.60 (m, 2H, H–2/H–2'), 2.09–2.32 (m, 8H, H–6/H–4/H–6'/H–4'), 1.95–2.02 (m, 4H, H–5/H–5'); EIMS: *m/z* 328 (6), 310 (9), 254 (4), 215 (40), 146 (26), 118 (58), 84 (77), 55 (100); $C_{19}H_{20}O_5$ (328.35): calcd. C 69.50; H 6.14. found: C 69.54; H 6.02%.

### 2-Methoxyphenyl-2,2′-methylenebis-(cyclohexane-1, 3-dione), 7

White solid; mp: 205–207 ºC; Yield: 96%; TLC: $R_f$ 0.51 (9:1 $CH_2Cl_2/CH_3OH$); UV (CH3OH): $\lambda_{max}$ 274 (log ε = 3.9) nm; IR (KBr): ν = 3023, 1613, 1604, 1511, 1461, 1339, 842 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 7.15–7.28 (m, 2H, H–5″/H–4″), 6.87 (td, *J* = 7.9 Hz, *J* = 1.8 Hz, 1H, H–4″), 6.79 (d, *J* = 7.9 Hz, 1H, H–6″), 5.33-5.50 (m, 1H, H–7), 3.71 (s, 3H, $OCH_3$), 3.72–3.80 (m, 2H, H–2/H–2′), 2.13–2.40 (m, 8H, H–4/H–6/H–4′/H-6′), 1.91–1.96 (m, 4H, H–5/H–5′); EIMS: *m/z* 342 (7), 243 (9), 229 (10), 199 (100), 171 (10), 139 (31), 84 (37), 55 (60); $C_{20}H_{24}O_5$ (342.38): calcd. C 70.16; H 6.48. found: C 69.89; H 6.21%.

### 4-Methoxyphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 8

White solid, m.p. 196-198 ºC; Yield: 96%; TLC: $R_f$ 0.56 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 301 (log ε = 3.4) nm; IR (KBr): ν = 3023, 1615, 1609, 1510, 1472, 1343, 841 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 6.99 (d, *J* = 8.4 Hz, 2H, H–2″/H–6"), 6.75 (d, *J* = 8.4 Hz, 2H, H–3″/H-5″), 5.12–5.29 (m, 1H, H–7), 3.77 (s, 3H, $OCH_3$), 2.58-2.75 (m, 2H, H–2/H-2′), 2.26–2.45 (m, 8H, H-4/H-6/H-4′/H-6′), 1.89–1.94 (m, 4H, H–5/H–5′); EIMS: *m/z* 342 (13), 324 (4), 243 (7), 229 (48), 199 (26), 145 (14), 117 (19), 84 (100), 55 (92); $C_{20}H_{22}O_5$ (342.38): calcd. C 70.16; H 6.48. found: C 68.92; H 6.19%.

### 3,4-Dimethoxyphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 9

White solid, m.p. 240-242 ºC; Yield: 92%; TLC: $R_f$ 0.52 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 308 (log ε = 3.4) nm; IR (KBr): ν = 3011, 1615, 1606, 1515, 1474, 843 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 6.85 (d, *J* = 8.4, 1H, H–5″), 6.68 (d, *J* = 1.3 Hz, 1H, H–2″), 6.58 (dd, *J* = 8.4 Hz, *J* = 1.3 Hz, 1H, H–6″), 5.17–5.26 (m, 1H, H–7), 3.78 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 2.47–2.55 (m, 2H, H–2/H–2′), 2.33–2.42 (m, 8H, H–4/H–6/H–4′/H–6′), 1.91–1.97 (m, 4H, H–5/H–5′); EIMS: *m/z* 372 (13), 354 (10), 323 (20), 260 (35), 229 (35),

84 (70), 55 (100); $C_{21}H_{24}O_6$ (372.00): calcd. C 67.73; H 6.50. found: C 67.32; H 6.24%.

**2-Ethoxyphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 10**

White solid, m.p. 173-175 ºC; Yield: 93%; TLC: $R_f$ 0.50 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 301 (log ε = 3.6) nm; IR (KBr): ν = 3002, 1615, 1606, 1515, 1473, 1340, 842 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.15–6.87 (m, 5H, Ar-H), 5.08-5.20 (m, 1H, H-7), 3.76 (q, *J* = 6.1 Hz, 2H, $OCH_2$), 2.48–2.56 (m, 2H, H–2/H-2′), 2.12–2.22 (m, 8H, H–4/H–6/H–4′/H–6′), 1.92–1.98 (m, 4H, H–5/H–5′); EIMS: *m/z* 356 (8), 272 (13), 254 (12), 215 (16), 199 (100), 145 (17), 55 (59); $C_{21}H_{24}O_5$ (356.41): calcd. C 70.77; H 6.79. found: C 69.98; H 6.37%.

**4-Ethoxyphenyl-2,2′-methylenebis-(cyclohexane-1, 3-dione), 11**

White solid, m.p. 162–164 ºC; Yield: 93%; TLC: $R_f$ 0.50 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 301 (log ε = 3.6) nm; IR (KBr): ν = 3002, 1616, 1602, 1514, 1477, 1340, 841 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.01 (d, *J* = 8.1 Hz, 2H, H–2″/H-6″), 6.65 (d, *J* = 8.1 Hz, 2H, H–3″/H–5″), 5.12–5.25 (m, 1H, H–7), 3.77 (q, *J* = 6.1 Hz, 2H, $OCH_2$), 2.48–2.56 (m, 2H, H–2/H–2′), 2.12–2.22 (m, 8H, H–4/H–6/H–4′/H-6′), 1.92–1.98 (m, 4H, H–5/H–5′); EIMS: *m/z* 356 (33), 321 (3), 282 (6), 244 (36), 215 (50), 199 (23), 84 (100), 55 (87); $C_{21}H_{24}O_5$(356.41): calcd. C 70.77; H 6.79. found: C 70.33; H 5.94%.

**4-N,N-Dimethylaminophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 12**

White solid, m.p. 252–254 ºC; Yield: 93%; TLC: $R_f$ 0.51 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 261 (log ε = 3.3) nm; IR (KBr): ν = 3029, 1621, 1615, 1501, 1312, 851 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 6.97 (d, *J* = 8.4 Hz, 2H, H–3″/H–5″), 6.75 (d, *J* = 8.4 Hz, 2H, H–2″/H–6″), 4.99–5.24 (m, 1H, H–7), 2.59–2.67 (m, 2H, H–2/H–2′), 2.89 (s, 6H, $N(CH_3)_2$), 2.17–2.39 (m, 8H, H–4/H–6/H–4′/H–6′), 1.91–1.97 (m, 4H, H–5/H–5′); EIMS: *m/z* 355 (6), 335 (20), 319 (12), 279 (10), 216 (50), 160 (6), 121 (13), 83 (49), 55 (100); $C_{19}H_{25}NO_4$ (355.00): calcd. C 70.96; H 7.09; N 3.94. found: C 71.25; H 7.02; N 3.68%.

**2-Chlorophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 13**

White solid, m.p. 198–200 ºC; Yield: 93%; TLC: $R_f$ 0.48 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 295 (log ε = 3.9) nm; IR (KBr): ν = 3020, 1615, 1612, 845 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.29 (d, *J* = 7.5 Hz, 1H, H-3″), 7.10 (dd, *J* = 7.5, *J* = 2.0 Hz, 1H, H–5″), 7.05 (t, *J* = 7.5 Hz, 1H, H-4″), 6.92 (d, *J* = 7.5 Hz, 1H, H–6″), 4.70–4.85 (m, 1H, H–7), 2.43–2.54 (m, 2H, H–2/H–2′), 2.12–2.41 (m, 8H, H–4/H–6/H-4′/H–6′), 1.89–1.95 (m, 4H, H–5/H–5′); EIMS: *m/z* 346 (8), 311 (5), 227 (15), 199 (100), 171 (10), 84 (30), 55 (40); $C_{19}H_{19}ClO_4$ (346.80): calcd. C 65.80; H 5.52. found: C 65.24; H 5.18%.

**3-Chlorophenyl-2,2′-methylenebis-(cyclohexane-1, 3-dione), 14**

White solid, m.p. 202–204 ºC; Yield: 92%; TLC: $R_f$ 0.50 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 301 (log ε = 4.0) nm; IR (KBr): ν = 3022, 1625, 1618, 1521, 1315, 842, 612 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.29 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H, H-4″), 7.21 (d, *J* = 1.5 Hz, 1H, H–2″), 7.11 (td, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H, H–5″), 7.12 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H, H-6″), 5.03–5.14 (m, 1H, H–7), 2.59–2.67 (m, 2H, H-2/H–2′), 2.17–2.39 (m, 8H, H–4/H–6/H-4′/H-6′), 1.91–1.97 (m, 4H, H–5/H–5′); EIMS: *m/z* 346 (24), 302 (12), 272 (9), 247 (19), 233 (20), 199 (59), 136 (40), 115 (41), 115 (22), 101 (34), 55 (100); $C_{19}H_{19}ClO_4$ (346.80): calcd. C 65.80; H 5.52. found: C 65.39; H 5.20.

**3-Bromophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 15**

White solid, mp: 203-205 ºC; Yield: 92%; TLC: $R_f$ 0.50 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 2.93 (log ε = 3.8) nm; IR (KBr): ν = 3029, 1622, 1616, 1509, 1312, 850, 608 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.58 (dd, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H–4″), 7.42 (d, *J* = 1.7 Hz, 1H, H–2″), 7.25 (td, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H–5″), 7.17 (dd, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H–6″), 5.10-5.20 (m, 1H, H–7), 2.58–2.69 (m, 2H, H–2/H–2′), 2.17–2.39 (m, 8H, H–4/H–6/H-4′/H–6′), 1.89-1.95 (m, 4H, H–5/H–5′); EIMS: *m/z* 391 (10), 279 (15), 200 (15), 199 (100), 182 (23), 143 (24), 128 (35), 102 (34), 84 (84), 55 (76); $C_{19}H_{19}BrO_4$ (391.25): calcd. C 58.33; H 4.89. found: C 57.97; H 4.43.

**3-Bromo-4-hydroxyphenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 16**

White solid, m.p. 292-294 ºC; Yield: 94%; TLC: $R_f$ 0.45 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 299 (log ε = 3.8) nm; IR (KBr): ν = 3012, 1612, 1618, 1512, 1475, 840 $cm^{-1}$; $^1H$-NMR (400 MHz, $CD_3OD$): $\delta$ = 7.18 (d, *J* = 1.5, 1H, H-2″), 6.80 (dd, *J* = 8.4 Hz, *J* = 8.4 Hz 1H, , H–6″), 6.70 (d, *J* = 8.4 Hz, 1H, H–5″), 5.12–5.22 (m, 1H, H–7), 2.49–2.55 (m, 2H, H–2/H–2′), 2.32–2.42 (m, 8H, H-4/H–6/H–4′/H-6′), 1.96-2.01 (m, 4H, H–5/H–5′). – EIMS: *m/z* 407 (36), 322 (77), 243 (59), 211 (100), 187 (39), 83 (100), 55 (74); $C_{19}H_{197}BrO_5$ (407.25): calcd. C 56.03; H 4.70. found: C 56.26; H 4.52.

**2-Nitrophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 17**

White solid, m.p. 210-212 ºC; Yield: 94%; TLC: $R_f$ 0.49 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 302 (log ε = 3.7) nm. – IR (KBr): ν = 3021, 1652, 1615, 1596, 841 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$): $\delta$ = 7.99 (dd, *J* = 8.1 Hz, *J* = 1.2 Hz, 1H,

H-5″), 7.56 (d, *J* = 8.1 Hz, 1H, H-3″), 7.32 (d, *J* = 8.1 Hz, 1H, H-4″), 7.32 (dd, *J* = 8.1 Hz, *J* = 1.2 Hz, 1H, H-6″), 4.90–5.08 (m, 1H, H–7), 2.52–2.60 (m, 2H, H–2/H–2′), 2.13–2.33 (m, 8H, H–4/H–6/H–4′/H–6′), 1.89–1.95 (m, 4H, H–5/H–5′); EIMS: *m/z* 357 (5), 322 (10), 230 (15), 199 (36), 144 (8), 84 (30), 55 (100); $C_{19}H_{19}NO_6$ (357.57): calcd. C 63.86; H 5.36; N 3.92. found: C 63.38; H 5.05; N 3.49%.

**3-Nitrophenyl-2,2′-methylenebis-(cyclohexane-1, 3-dione), 18**

White solid, m.p. 208-210 ºC; Yield: 91%; TLC: $R_f$ 0.50 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 307 (log ε = 4.07) nm; IR (KBr): ν = 3023, 1651, 1615, 1590, 1362, 841 $cm^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 8.11 (d, *J* = 8.4 Hz, 1H, H-4″), 7.22 (d, *J* = 1.8 Hz, 1H, H-2″), 7.10 (td, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H, H–5″), 6.87 (dd, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H, H–4″), 4.91–5.01 (m, 1H, H–7), 2.71–2.79 (m, 2H, H–2/H–2′), 2.05–2.40 (m, 8H, H–4/H–6/H–4′/H–6′), 1.91–1.97 (m, 4H, H–5/H-5′); EIMS: *m/z* 357 (10), 340 (5), 245 (15), 228 (36), 198 (15), 161 (15), 84 (77), 55 (100); $C_{19}H_{19}NO_6$ (357.57): calcd. C 63.86; H 5.36; N 3.92. found: C 63.28; H 5.12; N 3.82%.

**4-Nitrophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 19**

White solid, m.p. 219-220 ºC; Yield: 93%; TLC: $R_f$ 0.49 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 310 (log ε = 3.9) nm; IR (KBr): ν = 3023, 1670, 1623, 1511, 1366, 1344, 1465, 848 $cm^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 8.11 (d, *J* = 8.7 Hz, 2H, H–3″/H–5″), 7.26 (d, *J* = 8.7 Hz, 2H, H-2″/H-6″), 5.10–5.19 (m, 1H, H–7), 2.71–2.78 (m, 2H, H–2/H–2′), 2.05–2.40 (m, 8H, H–4/H–6/H–4′/H–6′), 1.88-1.93 (m, 4H, H–5/H–5′); EIMS: *m/z* 357 (5), 339 (4), 273 (11), 244 (8), 228 (40), 198 (23), 84 (79), 55 (100); $C_{19}H_{19}NO_6$ (357.35): calcd. C 63.86; H 5.36; N 3.92. found: C 62.83; H 5.12; N 3.47%.

**4-Chloro-3-nitrophenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 20**

White solid, m.p. 222–224 ºC; Yield: 93%; TLC: $R_f$ 0.43 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 310 (log ε = 3.9) nm; IR (KBr): ν = 3023, 1670, 1621, 1515, 1361, 1344, 1465, 842 $cm^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 8.15 (d, *J* = 8.7 Hz, H 2H, -3″/H–5″), 7.21 (d, *J* = 8.7 Hz, 2H, H–2″/H-6″), 5.10–5.19 (m, 1H, H–7), 2.71–2.78 (m, 2H, H–2/H–2′), 2.05–2.40 (m, 8H, H–4/H–6/H–4′/H–6′), 1.88–1.93 (m, 4H, H–5/H–5′); EIMS: *m/z* 391 (5), 356 (8), 279 (15), 244 (12), 217 (10), 163 (23), 135 (30), 55 (100); $C_{19}H_{19}NO_6$ (391.80): calcd. C 58.24; H 4.63; N 3.57. found: C 57.89; H 4.31; N 3.24%.

**α-Bromostyrenyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 21**

White solid, m.p. 150-152 ºC; Yield: 97%; TLC: $R_f$ 0.51 (9:1 $CH_2Cl_2/CH_3OH$); UV ($CH_3OH$): $\lambda_{max}$ 315 (log ε = 3.7) nm; IR (KBr): ν = 3012, 1615, 1541, 1515, 1475, 845 $cm^{-1}$; $^1$H–NMR (400 MHz, $CD_3OD$): $\delta$ = 6.85 (d, *J* = 8.3 Hz, 2H, H-2″/ H–6″), 6.79 (t, *J* = 8.3 Hz, 1H, H–4″), 6.68 (s, 1H, H–7″), 6.55 (t, *J* = 8.3 Hz, 2H, H-3″/H–5″), 5.18–5.30 (m, 1H, H–7), 2.47–2.53 (m, 2H, H–2/H–2′), 2.32–2.42 (m, 8H, H–4/H–6/H–4′/H-6′), 1.90–1.96 (m, 4H, H–5/H–5′); EIMS: *m/z* ; 417 (5), 308 (4), 225 (100), 197 (8), 165 (15), 141 (25), 127 (23), 55 (57); $C_{21}H_{21}BrO_4$ (417.29): calcd. C 60.44; H 5.07. found: C 60.19; H 4.78%.

**5-Methylfuranyl-2,2′-methylenebis-(cyclohexane-1,3-dione), 22**

White solid, m.p. 204–206 ºC; Yield: 96%; TLC: $R_f$ 0.49 ($CH_2Cl_2/CH_3OH$, 9:1); UV ($CH_3OH$): $\lambda_{max}$ 214 (log ε = 3.5) nm; IR (KBr): ν = 3012, 1615, 1605, 1518, 1475, 845 $cm^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 5.85 (d, *J* = 2.4 Hz, 1H, H-3″), 5.73 (d, *J* = 2.4 Hz, 1H, H–4″), 5.15–5.28 (m, 1H, H–7), 2.48–2.58 (m, 2H, H–2/H-2′), 2.33–2.42 (m, 8H, H–4/H–6/H–4′/H–6′), 1.91–1.97 (m, 4H, H–5/H-5′); EIMS: *m/z* 316 (7), 298 (75), 255 (100), 200 (40), 115 (40), 83 (36), 55 (53); $C_{18}H_{20}O_5$ (316.34): calcd. C 68.34; H 6.37. found: C 67.93; H 6.11%.

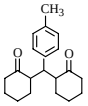
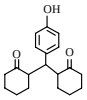
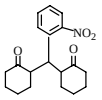
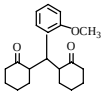
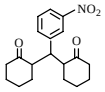
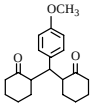
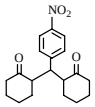
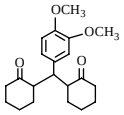
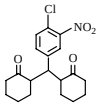
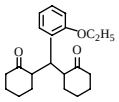
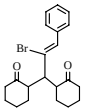
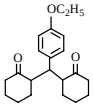
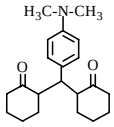
## DISCUSSION

The order of the lipoxygenase inhibiting activity of the compounds synthesized was 20 (18.5 μM) > baicalein (positive control, 22.4 μM) > 8 (24.3 μM) > 21 (32.6 μM) > 11 (33.3 μM) > 10 (34.5 μM) > 22 (34.9 μM) > 9 (35.6 μM) > 6 (40.0 μM) > 17 (41.6 μM) > 5 (47.3 μM) > 18 (48.6 μM) > 16 (49.1 μM) > 4 [56.2 μM] (Table 1). Compounds **2, 3, 7, 12, 13, 14, 15** and **19** have no inhibitory activity on the enzyme.

In our continuing research on biologically active compounds for use in medicine to treat inflammation, we hereby report on an alternate synthetic reagent, $Et_4NBr/NH_4Cl$, for the synthesis of tetraketones. It serves as a phase transfer reagent which not only increases the surface area of the reaction mixture but also enhances the water solubility of starting materials. This effect resulted in mild reaction conditions and a significant increase in the percentage yields of the target compounds compared to the previously reported protocols (2, 3). Polyoxygenated compounds have previously been reported as lipoxygenase inhibitors (8, 9). However, in the above method we have synthesized polyphenolics, polyoxygenated and other tetraketones.

From the above order of activity, the compounds derived from mesomerical electron donating groups *eg* 4, 5,

Table 1*: In vitro* lipoxygenase inhibition activity

| Compounds | Structures | Lipoxygenase $IC_{50}$ (μM) | Compounds | Structures | Lipoxygenase $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **2** |  | >500 | **13** |  | >500 |
| **3** |  | >500 | **14** |  | >500 |
| **4** |  | 56.2 | **15** |  | >500 |
| **5** |  | 47.3 | **16** |  | 49.1 |
| **6** |  | 40.0 | **17** |  | 41.6 |
| **7** |  | >500 | **18** |  | 48.6 |
| **8** |  | 24.3 | **19** |  | >500 |
| **9** |  | 35.6 | **20** |  | 18.5 |
| **10** |  | 34.5 | **21** |  | 32.6 |
| **11** |  | 33.3 | **22** |  | 34.9 |
| **12** |  | >500 | | | |

**Standard:** Baicalein ($IC_{50}$ = 22.4 μM)

6, 8, 9, 10 and 11 suggested that para-substituted compounds showed higher activity compared to substituents present at ortho or meta position.

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